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THE SIGNIFICANCE OF AUTOLOGOUS PLATELETS LABELLED WITH INDIUM-111 OXINATE IN PRIMARY IMMUNE THROMBOCYTOPENIA OF ADULTS AND CHILDREN

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Summary

The method of autologous platelet labelling with In-111 oxinate, and labelled platelets lifespan, production and sequestration site determination at the Center of Nuclear Medicine, Clinical Center of Serbia is the result of 24 years of personal experience in Belgrade and modification and improvement of the original method.

The improved method is described, and the significance of platelet lifespan, sequestration site and production investigation in 555 patients aged from 3,1-83 years, with clinical diagnosis of primary immune thrombocytopenia (ITP), as well as in 6 control healthy subjects is demonstrated.

On the basis of the obtained results, it was possible to confirm diagnosis of ITP in 477 (392 adults and 85 children) out of 555 patients (86 %) with clinical diagnosis of ITP. In 71 patient (69 adults and 2 children) out of 555 (13 %) ineffective platelet production was discovered as the mechanism of thrombocytopenia (patients had normal platelet lifespan, and ITP was excluded), and there were 7 patients (1%) with pseudothrombocytopenia (they had also normal platelet lifespan).

Seventy eight ITP patients were splenectomized after labelled platelets investigation. In all patients (100 %) with spleen or predominantly spleen as platelet sequestration site, splenectomy results were good. In patients with mixed platelet sequestration (in the spleen and liver), splenectomy results were good in 71 %. In ITP patients with hepatic platelet sequestration good splenectomy result was registered in only 20 %, while splenectomy failed in 80 % of patients. On the basis of labelled platelets sequestration site determination, it was possible to predict the efficacy of splenectomy in ITP patients.

This investigation enabled visualization of 18 accessory spleens in 13 ITP patients (in 2,7 % of ITP patients) and their role in thrombocytopenia could be estimated.

Unstable atherosclerotic plaques and acute thrombosis were visualized in ITP patients. Chronic thrombosis could not be registered because of the finished thrombus formation and no more platelet deposition.

Keywords: ^{111}In-oxinate labelled platelets, immune thrombocytopenia, splenectomy result prediction

INTRODUCTION

The history of platelet labelling goes back to 1956 when Leeksma and Cohen used radionuclide phosphorus-32 incorporated into diisopropylfluorophosphonate (^{32}P-DFP) for that purpose [1]. Only platelet (Pt) lifespan (without Pt production and sequestration site) could be determined, due to the physical characteristics of ^{32}P. Aas and Gardner in 1958 [2], and Najean in 1959 [3] introduced chromium-51 in the form od sodium-chromate (^{51}Cr-Na_2CrO_4) for platelet labelling. Both Pt lifespan and sequestration site could be estimated, but no images were available due to the low gamma photon yield and non-optimal gamma photon energy of ^{51}Cr for detection with nuclear medicine equipment. Contemporary method of Pt labelling is related to Thakur [4] and indium-111 (^{111}In), which offered good quality images. In Serbia ^{111}In-oxinate was introduced for the purpose of Pt labelling, Pt lifespan, Pt production and Pt sequestration site determination in 1991, at Center of Nuclear Medicine in Belgrade, and first results were published in 1993 [5-12].

Improved platelet separation and labelling procedure

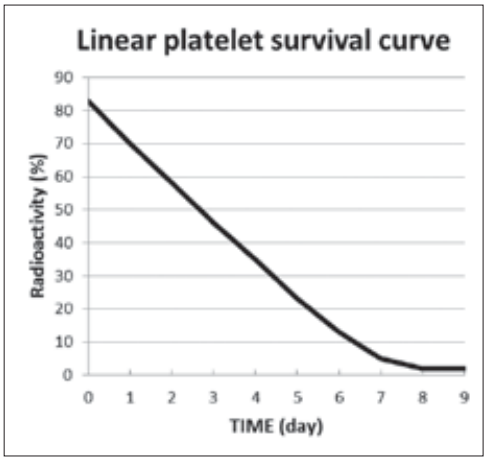
In order to be labelled, platelets have to be separated from the 60 ml of the whole blood sample, by means of differential centrifugation: two slow (at 150 g during 14 minutes first and 12 minutes second, with 1 minute acceleration and 2 minutes slowing down, enabling Pt rich plasma layer to be collected) and one fast centrifugation (of the Pt rich plasma at 640 g during 14 minutes, with additional 1 minute acceleration and 2 minutes slowing down). ^{111}In-oxinate is added to the re-suspended platelet pellet (together with the Tampon Tris buffer) and incubation lasting 20 minutes follows. The washing procedure, using the acidified (up to PH 6,0-6,5) platelet poor plasma and centrifugation (at 640 g during 14 minutes) eliminate the unbound radioactivity. About 5 ml of spared Pt poor plasma (not used for washing procedure) is added to the re-suspended labelled Pt pellet. Quality control of Pt separation and labelling procedure follows: 1) general yield of labelling, GYL, indicating the percentage of bound, compared to the used radioactivity (in the control group mean value was 84 %, and in ITP group 69,1 %; the difference was due to lower Pt blood count in ITP group: 1-191 x 10^9/l, mean value 45 x 10^9/l, compared to 214-266 x 10^9/l, mean value 255 x 10^9/l in the control group); 2) differential yield of labelling, DYL, showing the percentage of radioactivity bound to: platelets themselves, RBC+WBC, plasma, compared to the total bound radioactivity (mean Pt DYL in controls was 98,3 % and in ITP patients 94,7 %; mean RBC+WBC DYL in ITP was 4,0 and mean plasma DYL in ITP was 1,3 %). In addition to laboratory quality control (GYL and DYL), *in vivo* quality control was performed, by acquiring initial dynamic study

lasting 20 minutes after ^{111}In-oxinate labelled Pt intravenous injection (40 images, 30 seconds each), which is the most sensitive parameter for slight platelet damage (leading to temporary initial labelled platelets augmented accumulation in the liver).

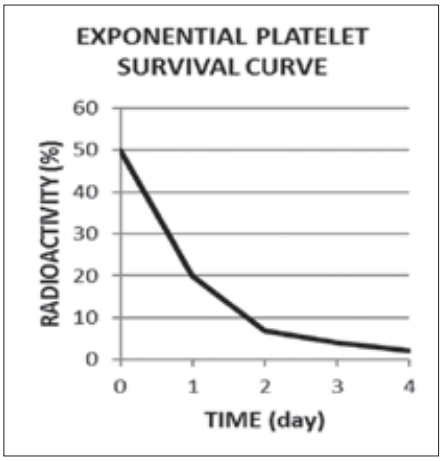
Investigation and results of platelet lifespan in 561 subjects

Platelet lifespan (Pt LS) was determined on the basis of the blood samples (3 ml each taken at 20 min., 2 h, 4 h after labelled platelets intravenous injection, and once a day till the day when blood radioactivity falls to about 10 % of the injected value) measurement in the gamma counter. Percentage of the circulating radioactivity (CR) was calculated for each moment of blood sample taking, using formula: CR=[(BR/3) / IR] x BV x 100 (BR, 3 ml blood sample radioactivity, IR, injected radioactivity, BV, blood volume of the investigated person expressed in ml), and the platelet survival curve was obtained. In the case of normal Pt lifespan, linear curve was obtained (fig. 1a) and platelet lifespan was read at the intercept of the time axis and the extrapolated platelet survival curve. In the case of shortened Pt lifespan, exponential curve was obtained (fig. 1b), and labelled Pt half disappearance time (T/2) expressed in days was determined, and Pt LS obtained using the formula: LS=(T/2) / ln2= 1,44 x (T/2).

Platelet LS in controls ranged 8-10 days (normal values: 7-10 days), mean value was 9 days, while in ITP patients it ranged from 0,7-228 hours (mean value was 1,6 days). Normal Pt LS excluded pathologic Pt destruction. Shortened Pt LS documented the presence and the degree of pathologic Pt destruction.



1a. Linear platelet survival curve.



1b. Exponential platelet survival curve.

Figure 1. Platelet survival curves

Determination and results of platelet production

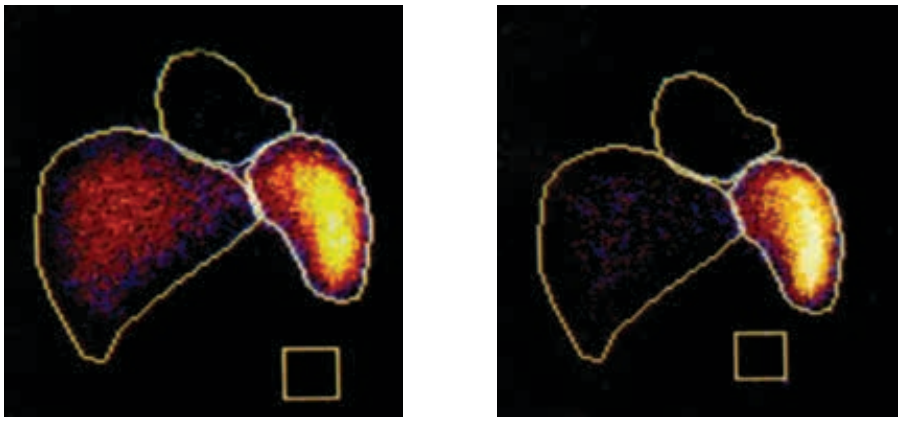
Platelet production index (PtPI) was calculated indirectly, using Pt LS and platelet blood count (BC) values of the investigated person (I) and healthy subject (H) in the case of the linear Pt survival curve: $PtPI_{lin} = (H_{LS} \times I_{BC}) / (I_{LS} \times H_{BC})$, where healthy subject Pt LS value is 8,5 days and Pt BC value is 300 x 10^9/l. For the exponential Pt survival curve, Pt production index ($PtPI_{exp}$) was calculated using Pt renewal (R) and BC values of healthy (H) and investigated (I) subject: $PtPI_{exp} = (I_R \times I_{BC}) / (H_R \times H_{BC})$. Platelet renewal was calculated according to the formula: R=69,3 / (T/2), where T/2 stands for labelled platelets half time (time in days when blood radioactivity falls to 50 % of the injected value). In the case when maximal circulating radioactivity (CR) was lower than 50 %, a correction factor (F) of $PtPI_{exp}$ was applied and multiplied with the value of PtPI. $F=50/CR_{max}$, where CR_{max} is the maximal circulating radioactivity (expressed in percents).

Platelet production index in controls ranged from 0,8 to 1,2 (mean value was 1,0) and in ITP patients from 0,1 to 28 (mean value was 1,4). Therefore, it was possible to distinguish two subgroups in our 477 ITP patients: with PtPI lower than 0,5 (48 patients, 10,1 %) and higher than 0,5 (429 patients, 89,9 %). In subgroup with lower PtPI, platelet lifespan was somewhat longer (3,4 days compared to 1,3 days) and Pt sequestration index lower (1,1 versus 1,6).

Platelet sequestration site determination and results

Platelet sequestration site (PtSS) was determined on the basis of sequential patient and test-tube with ^{111}In-oxinate imaging in anterior and posterior projection: at 20th minute, 2 h, 4 h after labelled Pt intravenous injection and thereafter once a day untill circulating radioactivity falls to less than 20 % of the injected value. Regions of interest (ROI) were outlined over the spleen, liver, heart and background radioactivity of the patient on anterior and posterior images, as well as over the test tube and its background. ROIs radioactivity values were corrected: a) for background, b) for gamma photons attenuation in the bed (on posterior projections only), c) for difference in the spleen and liver position (geometric mean of anterior and posterior projection radioactivity was calculated), d) for radioactive decay. PtSS was determined on the basis of platelet sequestration index (PtSI) value, calculated according to the formula: $PtSI = (S_t/L_t) / (S_0/L_0)$, where S_t is the radioactivity of the spleen at the time when more than 20% of circulating platelets disappeared from the circulation, L_t is the radioactivity of the liver at the same moment; S_0 is the radioactivity of the spleen at the first image obtained at 20th minute after labelled Pt intravenous injection, L_0 is the radioactivity of the liver at the same moment. In the case when initial labelled Pt kinetics was disturbed (during first 20 minutes after labelled Pt injection, on initial dynamic study), instead of the first image at 20th minute, the second image at 2h was used as the

reference time point for PtSI calculation. For PtSI higher than 2,0 PtSS was the spleen (fig. 2), for PtSI higher than 1,4 and lower or equal to 2, it was predominantly spleen, for PtSI values from 0,8-1,4 it was mixed, and for PtSI value lower than 0,8 it was the liver (fig. 3).

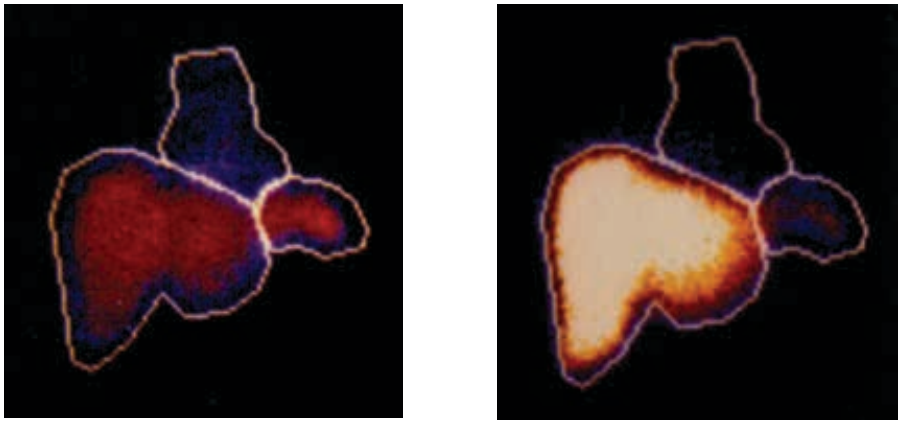


2a. **2b.**

Figure 2. Labelled platelets sequestration in the spleen

2a. First image of the ITP patient (obtained at 20th minute after labelled platelets intravenous injection) in anterior position with outlined regions of interest over the spleen, liver, heart and background. Splenic and hepatic tissues are visualized.

2b. Last image of the same patient. Only splenic tissue is visible.



3a. **3b.**

Figure 3. Labelled platelets sequestration in the liver

3a. First image of the ITP patient in anterior position with outlined regions of interest. Splenic and hepatic tissues are visualized.

3b. Last image of the same patient. Only hepatic tissue is visible.

Platelet SS in controls was the liver (in 4 subjects, 66,7 %) and mixed (liver and spleen equally, in two persons, 33,3 %). There was no splenic nor predominantly splenic platelet sequestration in the control subjects. On the contrary, in ITP patients platelet SS was the spleen or predominantly spleen in 50,4 %, mixed in 34,4 % and hepatic in 15,2 %.

Prediction of splenectomy results

Up till now we have information about seventy eight splenectomized (splenic tissue operatively removed) ITP patients after performing labelled Pt investigation. In all patients (100 %) with PtSS in the spleen or predominantly in the spleen, splenectomy results were good. In ITP patients with mixed Pt sequestration (in the spleen and in the liver equally), splenectomy results were good in 71 %. In ITP patients with hepatic Pt sequestration good splenectomy result was registered in only 20 %, while splenectomy failed in 80 %. Therefore, on the basis of labelled PtSS determination, it was possible to predict the efficacy of the splenectomy in our ITP patients. Splenectomy is advisable when PtSS is the spleen or predominantly spleen. On the contrary, in patients with hepatic Pt sequestration, splenectomy should be avoided. Our results are in agreement with the groups of Najean [13], Lamy (and Moisan) [14], Kinuya [15], Sarpatwari [16], Roca [17], while recent study of Navez [18] (in the group of 76 splenectomized ITP patients) claims that responce to splenectomy is independent of the site of Pt sequestration. Cuker [19] is suspicious about the relationship between PtSS and splenectomy result and advices labelled PtSS investigation only in the centers with extensive experience with this modality. The reason for discrepancies in the literature is in the non-uniformity of the methodology for PtSS determination, and therefore standardized methods offering best results are needed [19].

CONCLUSION

On the basis of the results of labelled platelets lifespan, production and sequestration site investigation, it was possible to confirm diagnosis of ITP in 477 (392 adults and 85 children) out of 555 patients (86 %) with clinical diagnosis of ITP. In 71 patient (69 adults and 2 children) out of 555 (13 %) ineffective platelet production was discovered as the mechanism of thrombocytopenia (patients had normal platelet lifespan, and ITP was excluded), and there were 7 patients (1%) with pseudothrombocytopenia (they had normal platelet lifespan, too).

This investigation enabled splenectomy result prediction in 65,6 % of ITP patients (with splenic and hepatic Pt sequestration site), visualization of 18 accessory spleens in 13 ITP patients (in 2,7 % of ITP patients)(6 in six patients investigated before splenectomy, and 12 in seven patients investigated after the splenectomy). Their role in thrombocytopenia could be estimated.

It was possible to detect unstable atherosclerotic plaques and acute thrombosis in ITP patients thanks to the images obtained after injection of labelled platelets. Focal zones of labelled platelets accumulation could be visualized at the places od unstable atherosclerotic plaques and acute thrombai. Chronic thrombosis could not be registered because of the finished thrombus formation and no more platelet deposition.

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Sažetak

Značaj autologno obilježenih trombocita indij-111 oksinatom u primarnoj imunološkoj trombocitopeniji u odraslih i djece

Metoda autolognog obilježavanja trombocita indij-111 oksinatom (^{111}In-oxinate), određivanje vijeka, produkcije i mjesta sekvestracije obilježenih trombocita u Centru za nuklearnu medicinu Kliničkog centra Srbije je rezultat 24-godišnjeg osobnog iskustva u Beogradu, modifikacije i usavršavanja originalne metode. Opisana je usavršena metoda, kao i značenje ispitivanja vijeka trombocita, proizvodnje trombocita i mjesta njihove sekvestracije u 555 bolesnika starosti 3,1 - 83 godine, sa kliničkom dijagnozom primarne imunološke trombocitopenije (ITP), kao i u 6 kontrolnih zdravih osoba.

Na temelju dobivenih rezultata, potvrđena je dijagnoza ITP u 477 (392 odrasle osobe i 85 djece) od 555 bolesnika (86 %) sa kliničkom dijagnozom ITP. U 71-og bolesnika (69 odraslih i 2 djece) od 555 (13 %) otkrivena je nedostatna proizvodnja trombocita kao mehanizam trombocitopenije (pacijenti su imali normalan vijek trombocita, pa je dijagnoza ITP isključena), a bilo je i 7 bolesnika (1%) sa pseudotrombocitopenijom (oni su također imali normalan vijek trombocita). Sedamdeset osam bolesnika sa ITP su splenektomirani nakon ispitivanja s obilježenim trombocitima. Rezultati splenektomije su bili dobri u svih bolesnika (100%) sa slezenom ili predominantno slezenom kao mjestom sekvestracije trombocita. U bolesnika s mješovitom sekvestracijom trombocita (u slezeni i jetri) rezultat splenektomije je bio dobar u 71 % operiranih bolesnika, a u bolesnika sa sekvestracijom trombocita u jetri u samo 20 %. Na osnovu određivanja mjesta sekvestracije obilježenih trombocita, bilo je moguće predvidjeti efikasnost splenektomije u bolesnika s ITP.

Ovo ispitivanje je omogućilo vizualni prikaz 18 akcesornih slezena u 13 ITP bolesnika (2,7 % ITP pacijenata), kao i procjenu njihove uloge u trombocitopeniji. Vizualno su prikazani nestabilni aterosklerotični plakovi i akutne tromboze u bolesnika sa ITP. Nisu se mogli utvrditi stari trombi, zbog toga što je kod njih završen proces formiranja (nema više nakupljanja trombocita).

Ključne riječi: Obilježeni trombociti ^{111}In-oksinatom, imunološka trombocitopenija, predviđanje rezultata splenektomije

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